

REMARKS

A. Status of the Claims

Claims 1-19 were pending in the case at the time of the Action and are currently under consideration. With this Response, claims 22-23 are newly added to more particularly point out and distinctly claim the invention.

B. Rejections Under 35 U.S.C. §103(a)

(1) Claims 1-19 are rejected under 35 U.S.C. §103(a) as being unpatentable over Martinell *et al.* (U.S. Patent 5,914,451) in view of Jefferson *et al.* (1987, *EMBO J.* 6:3901-3907). The Action asserts that Martinell *et al.* teaches transformation of soybeans, the use of meristematic tissue for transformation via particle bombardment, culturing transformed tissue to produce shoots, inducing root growth from transformed shoots, and selection in root medium with glyphosate. The Action also asserts that Jefferson *et al.* teaches assaying root tissue for the presence of reporter activity, detection of nucleic acid by Southern blot, and kanamycin selection using the NPTII gene. The Action further asserts that the claimed root assay does not provide any better results for determining germline transformation than would be expected. Applicants respectfully request reconsideration and withdrawal of this rejection in light of the following discussion.

The instant claims are directed to a method for early identification of germline transformed plants comprising steps of obtaining an extract of root tissue from transformed plant tissue and assaying the extract for the presence of the nucleic acid sequence, and identifying roots that assay positive for the nucleic acid sequence as putative germline transformed plants. The claimed invention is not obvious, at least, because the prior art fails to teach or suggest all

limitations of the claims. Neither Martinell *et al.* nor Jefferson *et al.* teach or suggest assaying roots to determine whether a plant is germline transformed, as recited in the instant claims. Additionally, the claimed methods are not simply obvious variants, providing predictable results, of the methods taught by the cited references, for the following reasons.

The claimed methods provide unexpected results and a solution to a long felt need. As discussed in the specification, plant transformation using meristematic tissues frequently creates chimeric plants that grow on selective medium but do not contain the transgene in germline tissues, such that the plant appears transgenic but the transgene is not passed to the seeds. The current invention is based in part on the surprising discovery that root tissue is a much better indicator of germline transformation than other tissues. Without being bound by any particular mechanism for this phenomenon, it is believed that the tissue type from which roots develop from transformed meristematic tissue is closely related to the tissues giving rise to reproductive tissues, such as found in flowers. The inventors further found that, by assaying an extract of root tissue, germline transformants could be identified at any stage after inducing root formation, with or without selection. This is significant because growth of roots on selection media can require up to four months (plants to maturity and seed harvest). Without the current methods using roots to identify germline-transformed plants, production of seeds would be required to identify germline transformation with high confidence. Thus, the instant invention is surprising because it was not previously known that roots are superior at reflecting germline status of the plant than other tissues. Additionally, the instant invention provides, for the first time, an early and accurate test for germline transformant status.

The significance of this discovery is further appreciated by considering the kanamycin and glyphosate selection regimes discussed in the specification. With glyphosate selection,

approximately 90% of the rooted plants are germline transformed (§0037 of the Specification [Publication US 2002/0123045]). However, many germline transformants resist rooting on glyphosate selection (§0039, specification). With the present invention, those plants resistant to rooting on glyphosate selection can be taken off selection and rooted. The roots of those plants can be tested for the presence of the transgenic gene/protein in the plant, which is likely to indicate the germline status of the plant. Without the instant assay, germline status could not be determined except after obtaining seeds of the plants.

When kanamycin selection is used approximately 50% of the rooted plants are germline transformed (§0037, specification). With the claimed methods, roots of those plants can be tested for the presence of the transgenic gene/protein in the plant, which is likely to indicate the germline status of the plant. This allows the elimination of all of the plants that tested negative using the root assay. Without the instant assay, germline status could not be determined except after obtaining seeds of the plants (resulting in a time savings of at least 4 months).

The Examples provide data supporting the claimed invention. Tables 2 and 3 (§0162 and 0164) show experimental results indicating 75-100% of the plants rooted under glyphosate selection were germline transformed and 67% of the plants rooted under kanamycin selection were germline transformed.

Table 4 (§165) provides experimental results showing that 34 of 34 plants that were rooted under glyphosate were positive using the root assay. Of those, 25 were germline positive, giving 74% correlation of the predicted germline positive using the root assay vs. actual germline positive. Table 4 also shows that 25 of 62 plants rooted under kanamycin selection were positive in the root assay. Of those 25, 16 were germline positive, giving a 70% correlation of the predicted vs. actual germline positive. In the case of the kanamycin selection, the root assay

allowed the elimination of 37 plants that were not germline transformed, which otherwise would have to be grown to seed to determine whether they were truly germline transformed.

Paragraph 0166 discloses results where plants under glyphosate selection were taken off selection to root. Of 125 such plants rooted off selection, 26 were positive with the root assay. Of those, 22 were germline positive (85% correlation between predicted vs. actual germline positive). Thus, the root assay allowed the elimination of 99 plants as potential transformants of plants rooted off of glyphosate selection that would otherwise require further growing and analysis to determine their germline status.

Example 3 (§§0167-0170) shows that the root assay correlated 91% of the time with petiole cross-section and pollen assays on cuttings from cotton plants raised from particle bombarded meristems. This further establishes the reliability of the assay.

Based on the above discussion, the claimed invention is surprising because there has been no previous indication that transformation status of roots correlates so well with germline status of the rooted plant. The claimed methods also address a long-felt need, providing a way of eliminating chimeras that are not germline transformed by assaying the vegetative plant, without requiring seed production.

Since neither Martinell *et al.* nor Jefferson *et al.* teach or suggest assaying roots to determine whether a plant is germline transformed, and since the assay is based in part on the unexpected finding that assaying roots of a rooted plant for transformation status correlates with the plant's germline transformation status, the instant invention is not obvious under 35 U.S.C. 103(a) based on Martinell *et al.* in view of Jefferson *et al.* Withdrawal of the rejection is respectfully requested.

(2) Claims 1-19 are also rejected under 35 U.S.C. §103(a) as being unpatentable over Firoozabady *et al.* (1987, *Plant Mol. Biol.* 10:105-116) in view of Jefferson *et al.* (1987, *EMBO J.* 6:3901-3907). The Action asserts that Firoozabady *et al.* teach transformation of cotton, the use of cotyledon tissues for transformation via *A. tumefaciens*, the use of NPTII/kanamycin selection, and culturing transformed tissues to germinate and form plantlets.

The Action also asserts that Jefferson *et al.* teaches assaying root tissue for the presence of reporter activity, detection of nucleic acid by Southern blot, and kanamycin selection using the NPTII gene, and that the claimed root assay does not provide any better results for determining germline transformation than would be expected. Applicants respectfully request reconsideration and withdrawal of this rejection in light of the following discussion.

The instant claims are directed to a method for early identification of germline transformed plants comprising steps of obtaining an extract of root tissue from transformed plant tissue and assaying the extract for the presence of the nucleic acid sequence, and identifying roots that assay positive for the nucleic acid sequence as putative germline transformed plants. The claimed invention is not obvious, at least, because the prior art fails to teach or suggest all limitations of the claims. Neither Firoozabady *et al.* nor Jefferson *et al.* teach or suggest assaying roots to determine whether a plant is germline transformed, as recited in the instant claims.

As discussed above in the context of the obviousness rejection based on the combination of Martinell *et al.* and Jefferson *et al.*, the claimed invention is surprising because there has been no previous indication that transformation status of roots in particular correlates with germline status of the rooted plant. The claimed methods also address a long-felt need, since they provide

a way of eliminating chimeras that are not germline transformed by assaying the vegetative plant, without seed production.

Since neither Firoozabady *et al.* nor Jefferson *et al.* teach or suggest assaying roots to determine whether a plant is germline transformed, and since the assay is based in part on the unexpected finding that assaying roots of a rooted plant for transformation status correlates with the plant's germline transformation status, the instant invention is not obvious under 35 U.S.C. 103(a) based on Firoozabady *et al.* in view of Jefferson *et al.* Withdrawal of the rejection is respectfully requested.

C. Obviousness-Type Double Patenting Rejection

In regard to the obviousness-type double patenting rejection, Applicants respectfully traverse but note that a Terminal Disclaimer will be submitted upon the allowability of the case. The rejection is therefore believed moot and removal thereof is thus respectfully requested.

D. Conclusion

In view of the foregoing, it is respectfully submitted that each of the pending claims is in condition for allowance, and a Notice of Allowance is earnestly solicited.

The Examiner is invited to contact the undersigned attorney at (214) 259-0932 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

/Ron J. Laby/

Reg. No. 53,173
Agent for Applicants

Sonnenschein Nath & Rosenthal LLP
1717 Main Street, Suite 3400
Dallas, TX 75201
Tel.: 214.259.0932
Fax: 214.259.0910
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